

ASSESSING NON-INVASIVE METHODS FOR DETERMINING LARVAL INSTARS

IN BUMBLE BEE





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INTRODUCTION

Bees are in decline globally due to human disturbances, yet the impacts of anthropogenic stressors on bee larvae remain understudied [1]. Bumble bees have been used as model organisms in lab research, and *in vitro* rearing techniques (i.e., 24-well plates) have been developed to assess the effects of stressors on larvae [2]. Bumble bee larvae undergo four distinct instars before pupation, and each instar may exhibit varying sensitivities to human perturbations [3].

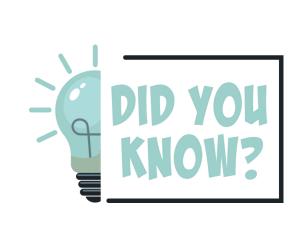


Figure 1. Larval instars in Bombus terrestris.

The conventional method for determining larval instar involves measuring head capsule width [4]. However, this technique is highly invasive, thereby limiting its applicability for *in vitro* settings. In this study, we aim to establish less-invasive alternatives for instar determination based:

- (i) on larval body area,
- (ii) on body mass,
- (iii) on head capsule width from sacrificial sampling within the same larval batch.

MATERIAL AND METHODS Larval sampling Colony worker Plate grafting of larvae from batches removal Freezing n = 533Nest batches Measurements Isolated cells Digital microscope Body mass Cell batch Batch of origin Head width Body area



To feed *Bombus* larvae *in vitro*, a drop of pollen-syrup mixture is applied to the larval abdomen, enabling individual feeding.



RESULTS AND CONCLUSION

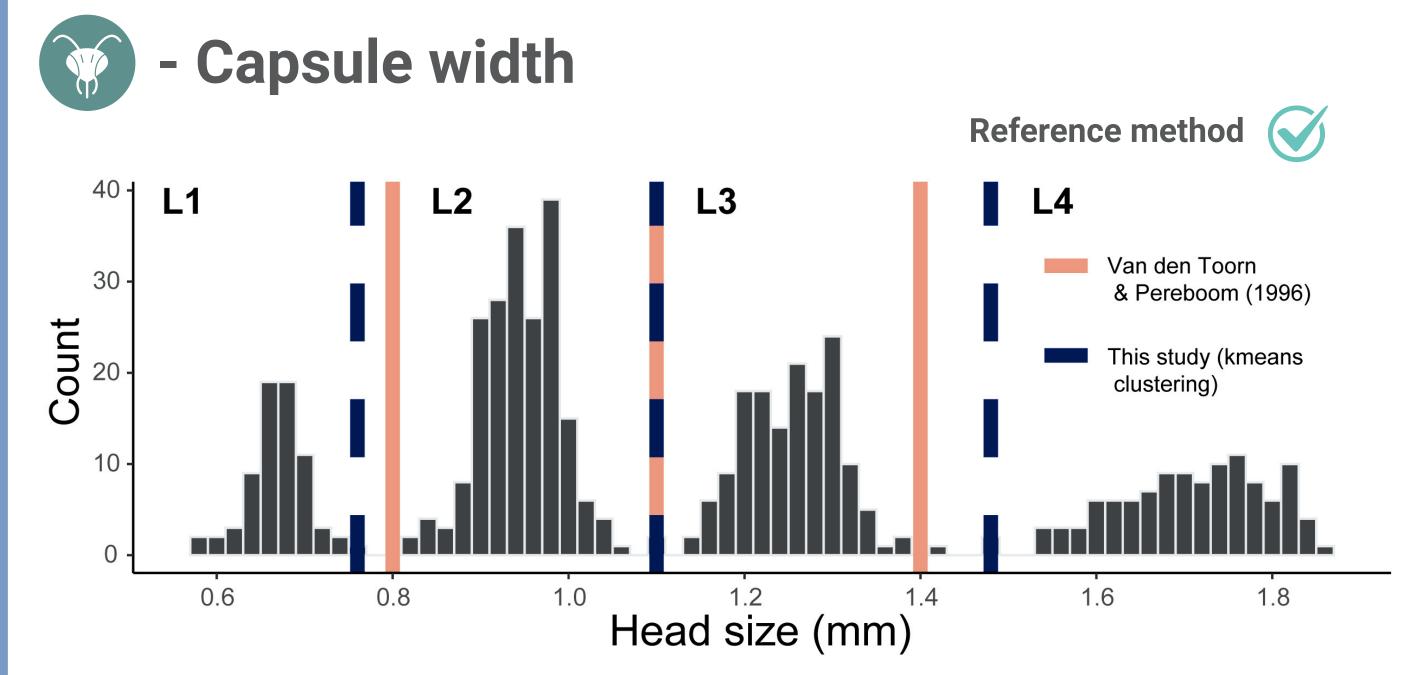


Figure 2. Distribution and clustering of the head capsule width of 553 *Bombus terrestris* larvae, showing well-defined clusters based on capsule width.

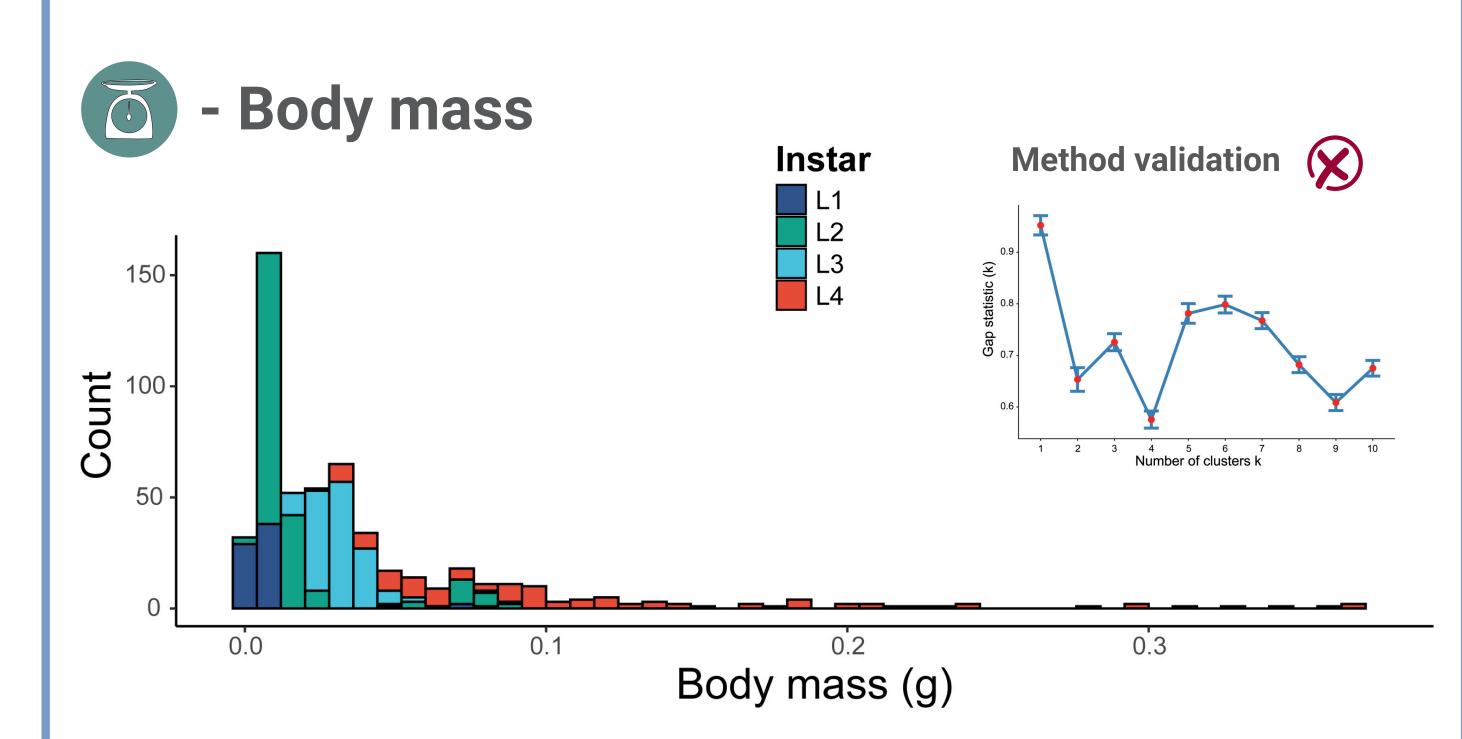


Figure 3. Distribution of body masses with instars highlighted, showing no well-defined clusters and number of clusters based on larval body masses.

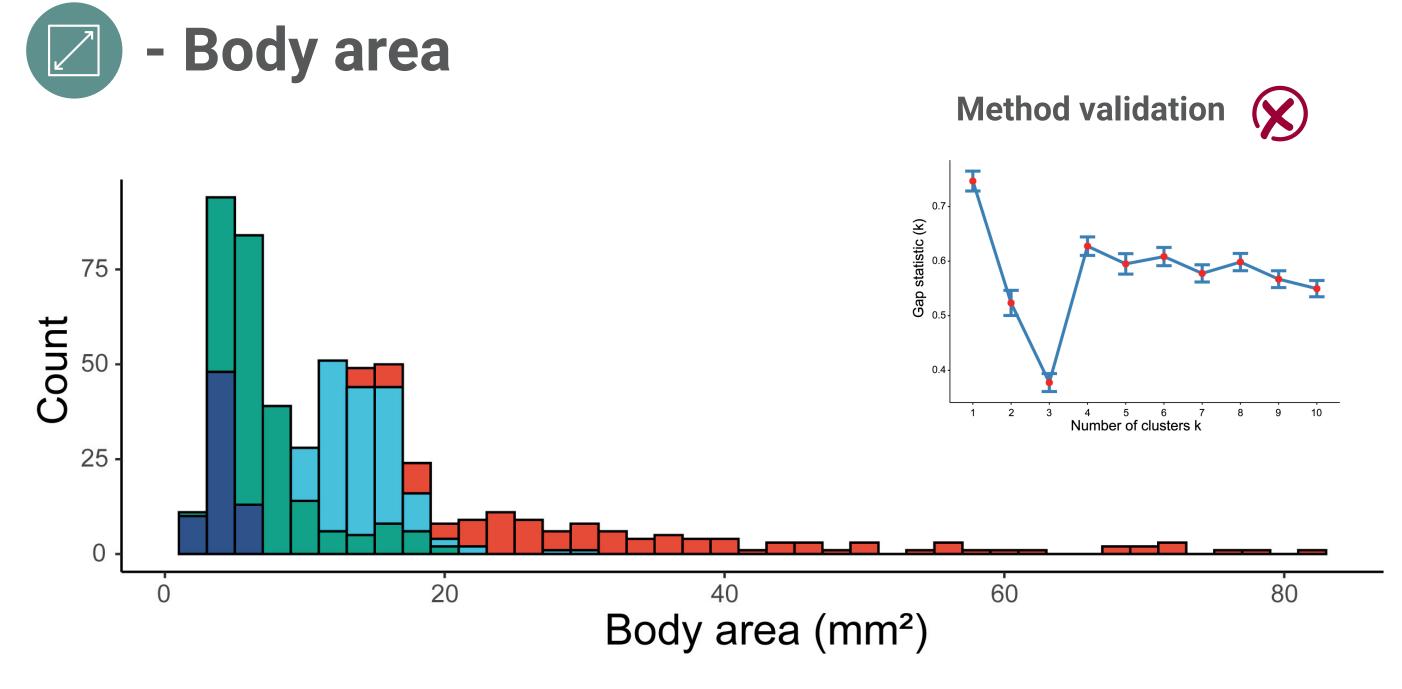


Figure 4. Distribution of body areas with instars highlighted, showing no well-defined clusters and number of clusters based on larval body areas.

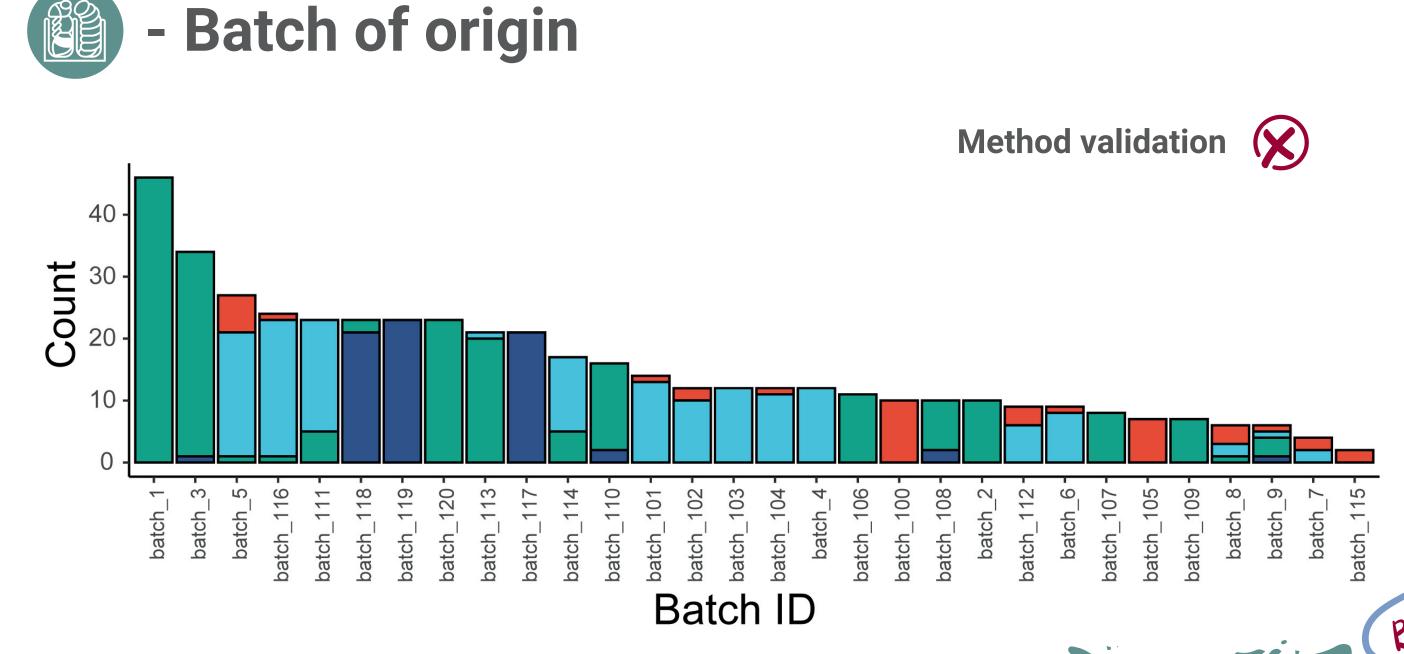


Figure 5. Larval instars found within batches. In virtually 60% of the cases, batches contained more than a single instar.





- [2] Gekière et al. (2025) Copper-accelerated pupation in larvae of the buff-tailed bumble bee. Apidologie. 56:4.
- [3] Tran et al. (2020) The effect of warming on pesticide toxicity is reversed between developmental stages in the mosquito C. pipiens. Sci. Total Environ. 717:134811.
- [4] Van den Toorn & Pereboom (1996) Determination of larval instar in bumblebees by using the head capsule width. *Proc. Exp. Appl. Entomol.* 7:65-69.

